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☐ Initial submission ☒ Revised version ☐ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Due to the high reproducibility and consistency between cell cultures, in in vitro studies it was predetermined that a sample size of at least n=2 would allow for adequate analysis to reach meaniful conclusions of the data. However, in in vivo studies, higher variance are seen, therefore, a higher set of samples is used to compensate for this natural variance.

2. Data exclusions

Describe any data exclusions.

No data was excluded from studies.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All replication of experiments was successful; however, in the case of replication studies of survivin expression from in vivo experiments (Fig. 4) replication was not fully possible due to small tumor volumes and limited materials.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Throughout the whole experiment, samples and animals were randomized into groups.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

No blinding was used throughout experiments. All data collected was quantifiable and blinding would not change any bias in data collected.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	AFM - FemtoscanOnline EM - FEI Software NTA - Malvern Nanoparticle Tracking Analysis 3.20 Zeta Potential & DLS - Zetasizer V7.11 Gel Images - Quant TL & ImageJ V1.50i Flow Cytometry - FlowJo 7.6.1 Confocal Microscopy - Fluoview (multiple versions)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Nanoparticles and exosomes used in these studies were made in house in the Peixuan Guo lab. Materials are available through requests to corresponding author via reasonable request. All other materials used are commercially available an company information is provided within manuscript.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Primary antibodies used for western blot analysis were rabbit anti-human survivin antibody (R&D system, AF886), rabbit anti-human β-actin (Abcam, ab198991), rabbit anti-human TSG101 (Thermo Scientific, PA5-31260), rabbit anti-human integrin α4 (Cell Signaling, 4711S), rabbit anti-human integrin α6 (Cell Signaling, 3750S), rabbit anti-human integrin β1 (Cell Signaling, 4706S), rabbit anti-human integrin β4 (Cell Signaling, 4707S), rabbit anti-human integrin β5 (Cell Signaling, 4708S), rabbit anti-human Glypican 1 (Thermo Fisher, PA5-28055), GAPDH antibody (Santa Cruz Biotechnology).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	HEK293T, KB, LNCaP-FGC, PC-3, MDA-MB-231, and MDA-MB-468 cells were obtained from ATCC, and LNCaP-LN3 cells were obtained from the MD Anderson Cancer Center.
b. Describe the method of cell line authentication used.	Cell cultures purchased from ATCC were authenticated by Short Tandem Repeat (STR) prior to purchase, and LNCaP-LN3 cells were authenticated prior to receiving the cells as a gift.
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were not tested for mycoplasma contamination.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	KB cell line has been listed as a misidentified cell line that has been derived by contamination of HeLa cells, it serves as an ideal model in these studies. KB cells are known to overexpress folate receptors, allowing for proper specific targeting through the use of folate on RNA nanoparticles. The derivation of the KB cell line will not affect its use as a model to test the folate receptor-targeting property of RNA-displaying EVs.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

6-8 week-old male nude mice (Nu/Nu) were purchased from Charles River (Wilmington, MA). 4- week-old female athymic nu/nu outbred mice were acquired from the athymic nude mouse colony maintained by the Target Validation Shared Resource at the Ohio State University; the original breeders (strain #553 and #554) for the colony were received from the NCI Frederick facility and were used for all studies. Male NOD-scid IL2Rgammanull mice were purchased from the Jackson Laboratory (Bar Harbor, ME).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No humans were used in studies.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Unchecked box above are not applicable to the data presented in this manuscript. Adherent cells used for flow cytometry studies were tripsinized and placed into suspension of blank cell culture medium with desired concentrations of fluorescently labeled-exosome samples. Cells were then incubated for 2 hours at 37 degree C as an exosome binding period. Cells were then washed in PBS to remove unbound fluorescent materials and passed through a screening filter to remove cell aggregates and then ran on flow looking at desired fluorophore signal.
6. Identify the instrument used for data collection.	Samples were analyzed by FACSCalibur with four lasers.
7. Describe the software used to collect and analyze the flow cytometry data.	All data was analyzed using FlowJo 7.6.2 with overlay histogram plots produced within FlowJo.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	During sample measurements and initial gate was used to ensure a cell count of 10,000 or 20,000 cells or events was collected of a relevant cell population.
9. Describe the gating strategy used.	Initial cell populations were gated for a live population using FSC and SSC plot of cell only sample. The gate was set to remove cell debris and dead cells (small FSC v SSC) and large clumps or aggregates of cells (large FSC or SSC) and used across all samples. This live population was then used in fluorescent histograms. No gating was applied to histograms.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒